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Carcinogenic Activity of Haloacetonitriles and Haloacetone Derivatives in the Mouse Skin and Lung

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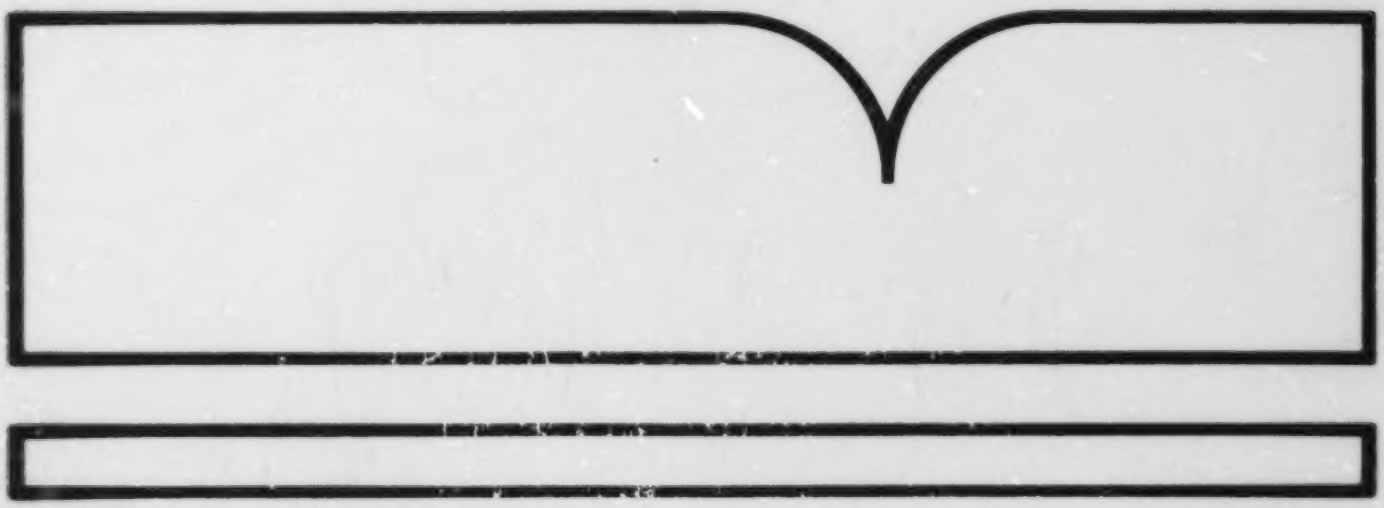

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CARCINOGENIC ACTIVITY OF HALOACETONITRILES AND HALOACETONE DERIVATIVES IN THE MOUSE SKIN AND LUNG

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16. ABSTRACT

Halogenated acetonitriles and acetones are common by-products of drinking water chlorination and have been identified in reactions with model substrates. Members of both classes have been shown to be mutagenic in Salmonella, to induce sister chromatid exchange in mammalian cells and/or to interact with DNA. Such results would imply a capability for these chemicals to act as carcinogens. The present study was undertaken to determine whether the members of these classes most often found in drinking water were capable of acting as carcinogens on the mouse skin or lung. Studies included the chloro-, dichloro-, trichloro-, bromochloro- and dibromo- substituted acetonitriles. The 1,1-dichloro- and 1,1,1-trichloro- substituted acetones were tested.

These data clearly show that the haloacetonitriles found in drinking water, with the possible exception of the dichloro-compound possess carcinogenic properties. To date, it has not been possible to demonstrate such activity with the chlorinated acetones. These findings emphasize the need to consider by-products other than the trihalomethanes when assessing carcinogenic hazards that are associated with alternative forms of drinking water disinfection.

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ABSTRACT

Halogenated acetonitriles and acetones are common by-products of drinking water chlorination and have been identified in reactions with model substrates. Members of both classes have been shown to be mutagenic in Salmonella, to induce sister chromatid exchange in mammalian cells and/or to interact with DNA. Such results would imply a capability for these chemicals to act as carcinogens. The present study was undertaken to determine whether the members of these classes most often found in drinking water were capable of acting as carcinogens on the mouse skin or lung. Studies included the chloro-, dichloro-, trichloro-, bromochloroand dibromo- substituted acetonitriles. The 1,1-dichloro- and 1,1,1-trichloro-substituted acetones were tested.

Significant increases in the yield of squamous cell papillomas and carcinomas were observed in the skin of Sencar mice treated topically with initiating doses of chloro-, bromochloro- and dibromoacetonitrile when followed by application of 1 µg of 12-O-tetradecanoyl-phorbol-13acetate three times weekly for 20 weeks. Dichloroand trichloroacetonitriles were considerably less effective as tumor initiators in the mouse skin. Single topical applications of 1,1-dichloroand 1,1,1trichloroacetone failed to produce increased yields of skin tumors under the same conditions. Administration of 10 mg/kg orally three times weekly over a period of eight weeks to strain A/J mice results in significantly elevated yields of lung adenomas at nine months of age with chloro-, trichlor- and bromochloro- acetonitrile. Marginal increases were seen with the dichloro and dibromosubstituted compounds.

These data clearly show that the haloacetonitriles found in drinking water, with the possible exception of the dichloro-compound possess carcinogenic properties. To date, it has not been possible to demonstrate such activity with the chlorinated acetones. These findings emphasize the need to consider by-products other than the trihalomethanes when assessing carcinogenic hazards that are associated with alternative forms of drinking water disinfection.

Chlorinated and brominated acetonitrile and acetone derivatives have been shown to be produced as a result of the chlorination of drinking water[1,2] or humic acids[3]. Haloacetonitriles have also been observed in the stomach contents of rats treated with aqueous solutions of chlorine[4] and result from reactions of chlorine with certain amino acids[5]. Since there have been a number of studies that associate the incidence of certain cancers with the chlorination of drinking water, it is important to assess the carcinogenic properties of products of chlorination.

The intent of the present paper is to review the evidence that haloacetonitrile and haloacetone derivatives that have been identified as by-products of chlorination possess biological properties that are associated with chemical carcinogens.

METHODS

The methodology utilized in these experiments have been published in detail elsewhere[6]. The mutagenic activity of these compounds was evaluated using the standard plate assay of[7] with and without a fraction of rat liver homogenate; by examining the induction of sister chromatid exchange in Chinese hamster ovary cells (CHO) *in vitro*, also with and without metabolic activation; by enumerating the number of micronuclei in polychromatic erthyrocytes obtained from the bone marrow of CD-1 mice treated *in vivo*; and by determining the

percentage of spermhead abnormalities at 1, 3 and 5 weeks following *in vivo* exposure of B6C3F1 mice. The chemicals were tested for carcinogenic activity as tumor initiators in the skin of female Sencar mice or by their ability to increase the yield of lung tumors in Strain A/J mice.

RESULTS

Table 1 summarizes the activity of the haloacetonitriles and haloacetone derivatives in *Salmonella typhimurium* strain TA100. In general, this is the strain that gives the greatest response to these. Of 5 haloacetonitriles tested two are positive in the Ames' test, dichloroacetonitrile (DCAN) and bromochloroacetonitrile (BCAN). Both chemicals gave evidence of activity in the absence of rat liver homogenate 9000 X g supernatant (S-9) in strain TA1535 whereas such direct action was evident only for DCAN in strains TA98 and TA100. The absence of direct acting activity with BCAN in TA100 is probably more of a technicality since there was a clear indication of increased activity in the absence of S-9 fraction, but the response failed to reach the 2-fold over background criteria used to designate a positive response. This appeared to result from the cytotoxic properties of BCAN. In point of fact, the initial slope of this response indicates slightly greater specific activity than that noted for BCAN with S-9 in TA100[6]. Six chlorinated acetones have been tested for mutagenic activity in Salmonella typhimurium, 1,1-dichloropropanone (1,1DCP), 1,3-dichloropropanone (1,3-DCP), 1,1,1-trichloropropanone (1,1,1-TCP), 1,1,3-trichloropropanone (1,1,3-TCP), 1,1,3, 3-tetrachloropropanone

(1,1,3, 3-TCP) and pentachloropropanone (PCP). The aldehydes 2-chloropropenal and 2,3,3-trichloropropenal, have been identified as by-products of the chlorination of humic acids[8],9 and are included in Table 1 for perspective. These chemicals are the most potent mutagens in Salmonella (Table 1) yet identified as a chlorination by-products. Of the 6 chlorinated acetone derivatives tested, 1,3-DCP was the most potent, followed by 1,1,3-TCP, 1,1,3,3-TCP, PCP, 1,1,1-TCP and 1,1-DCP, respectively. In all cases, the activity of these compounds was expressed in the absence of metabolic activation.

The haloacetonitriles have also been evaluated for their ability to induce sister chromatid exchange (SCE) in CHO cells (Table 2). As in the case with the Ames' test, the activity of the haloacetonitriles was clearly evident in the absence of S-9 fraction suggesting that these compounds do not require metabolic activation. The ability to induce SCE in CHO cells was seen to follow an orderly progression with the degree of chlorine substitution and was further enhanced with the substitution of bromine for chlorine.

Two attempts have been made to determine if the mutagenic activity of the haloacetonitriles could be demonstrated in vivo: Induction of micronuclei in polychromatic erythrocytes and spermhead abnormalities. None of the haloacetonitriles were found capable of inducing micronuclei in polychromatic erythrocytes isolated from the bone marrow of mice treated orally with 5 consecutive daily doses of

0, 12.5, 25 and 50 mg/kg[6]. The same dosing schedule failed to induce spermhead abnormalities in B6C3F1 mice sacrificed at either 1, 3 or 5 weeks following the last dose with all five haloacetonitriles[10].

Three of the five haloacetonitriles tested were clearly capable of acting as tumor initiators in the skin of Sencar mice (Table 3). In this case, the chemicals were applied topically in doses of 200, 400 and 800 mg/kg three times weekly for two weeks. Two weeks following the last dose of haloacetonitrile, the animals were subjected to a promotion schedule of topical applications of 12-O-tetradecanoyl-phorbol-13-acetate (TPA) for 20 weeks. DBAN, BCAN and CAN were all found capable of acting as tumor initiators under these conditions. DBAN was found to be the most potent followed by BCAN and CAN, respectively. The yield of squamous cell carcinomas at one year was also increased significantly with these three compounds which was roughly proportional to the benign tumor yield. TCAN significantly increased tumor yields at a total dose of 2400 mg/kg, however, 4800 mg/kg failed to produce a consistent response. DCAN tended to increase tumor yield in a dose-related manner, but the magnitude of the response must be considered marginal.

Table 4 provides data on the tumor initiating activity of haloacetone derivatives. 1,1-DCP and 1,1,1-TCP are the only members of this class which have been tested to date. Single oral doses of 200 mg/kg 1,1-DCP or 50 mg/kg 1,1,1-TCP or topical doses of 400 mg/kg for

both compounds. Twenty-four weeks following the start of the promotion schedule with TPA outlined above, there was no evidence of an increased yield of skin tumors as a result of these treatments.

The results of a preliminary evaluation of the haloacetonitriles to increase the lung tumor yield in strain A/J mice are provided in Table 5. In these experiments a single oral dose level, 10 mg/kg administered 3 times weekly for 8 weeks, was utilized. Although all of the haloacetonitriles increased the yield of lung tumors, only in the case of CAN, TCAN and BCAN were the increases statistically significant ($P<0.05$). Due to the relative ly large variation in the background levels of lung tumors in this strain, even these results should be interpeted with caution. However, it should be pointed out that 10 mg/kg is considerably below the maximally tolerated dose for these chemicals. They are currently being tested at higher doses.

DISCUSSION

It is clear from the above information that consideration of the carcinogenic hazards that may be associated with the chlorination of drinking water must go beyond formation of the trihalomethanes. At least two additional classes of chemicals must be considered, halogenated acetone and acetonitrile derivatives.

In the case of the haloacetone derivatives, testing has been limited to mutagenicity testing for most of the class. Carcinogenicity testing has been limited to two of the derivatives that are relatively weak mutagens, 1,1-DCP and 1,1,1-TCP. It is relatively

clear that substitution of chlorine on each of the two available carbon atoms considerably enhances the mutagenic potency. Consequently, even though the 1,3-substituted compounds are usually reported at lower concentrations, the fact that they are 2 to 4 orders of magnitude more potent than the corresponsing 1,1-derivatives, they should be subjected to a more thorough evaluation of their carcinogenic properties. For similar reasons, the extremely potent by-product 2-chloro-propenal deserves further attention as well. It also should be remembered that the biological properties beyond bacterial mutagenesis assays of the brominated derivatives of these two classes of by-products have yet to be considered.

The haloacetonitriles clearly possess carcinogenic as well as mutagenic properties. Although there does not appear to be a consistent relationship in the potency of these compounds in the various test systems to which they have been subjected, it appears that the brominated derivatives are the most potent carcinogens in the mouse skin. However, CAN and TCAN appear capable of inducing lung tumors in A/J Mice while CAN will also clearly act as a tumor initiator in the skin of the Sencar mouse. Only DCAN has failed to produce a carcinogenic response in tests conducted to date. The most critical issue that remains with the haloacetonitriles is an evaluation of their ability to produce cancer by a systemic route of administration. Although suggestive, the results which have been obtained to date on lung tumor induction in strain A/J mice are quite marginal. Preliminary information would suggest, however, that these compounds are capable of interacting

with DNA in vivo (Pereira, personal communication). Nevertheless, the only means of assessing the carcinogenic hazard these chemicals might pose for consumers of drinking water in quantitative terms are long term bioassays in experimental animals.

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Table 1. Mutagenic Activity of Haloacetonitriles and Chlorinated Acetone and Aldehyde Derivatives in Salmonella Typhimurium Strain TA100

Chemical	Net revertants/uMole[a]
Chloroacetonitrile (CAN)[b]	0
Dichloroacetonitrile (DCAN)[b]	7.1 x 10
Trichloroacetonitrile (TCAN)[b]	0
Bromochloroacetonitrile (BCAN)[b]	(8.6×10^2)
Dibromoacetonitrile (DBAN)[b]	0
1,1-Dichloropropanone (1,1-DCP)[c]	4.8×10^0
1,3-Dichloropropanone (1,3-DCP)[c]	1.4×10^4
1,1,1-Trichloropropanone (1,1,1,-TCP)[c]	9.3×10^2
1,1,3-Trichloropropanone (1,1,3-TCP)[d]	4.0×10^3
1,1,3,3-Tetrachloropropanone (1,1,3,3-TCP)[c]	1.5×10^3
Pentachloropropanone (PCP)[c]	8.5×10^2
2-Chloropropenal (2-CP)[e]	1.1×10^5
2,3,3-Trichloropropenal (2,3,3-TCP)[e]	2.2×10^5

[a] The net revertants/u Mole were calculated from the linear portion of the dose response curves. Numbers included within parenthesis indicate result in the presence of a 9000 x g supernatant of a rat liver homogenate.

[b] Based on data of Bull et al.,[6]

[c] Based on data of Meier et al.,[9]

[d] Based on data of Douglas et al.,[11]

[e] Based on data of Rosen et al.,[12]

Table 2. Induction of Sister Chromatid Exchange in Chinese Hamster Ovary Cells, *In Vitro*, by Haloacetonitriles

Chemical	Induced SCEs
Chloroacetonitrile	50 ± 11[a]
Dichloroacetonitrile	103 ± 26
Trichloroacetonitrile	230 ± 26
Bromochloroacetonitrile	396 ± 69
Dibromoacetonitrile	469 ± 17

[a] Based on data of Bull et al., (1984)

Table 3. Tumor Initiating Activity of Haloacetonitriles in the Skin of the Sencar Mouse.

Chemical	ED35[b]	% Animals with Squamous Cell Carcinomas at 2.4 g/kg
Chloroacetonitrile	2.9	10.8[c]
Dichloroacetonitrile	-	8.6
Trichloroacetonitrile	-	9.7
Bromochloroacetonitrile	2.2	18.9[c]
Dibromoacetonitrile	1.6	23.5[c]
Control	-	4.9

[a] Based on data of Bull et. al., (1984)

[b] Total dose in grams necessary to initiate papillomas in 35% of the animals.

[c] Overall response (including higher doses) is significantly different from control incidence $P<0.05$ using the Fisher Exact Test.

Table 4. Tumor Initiating Activity of Chlorinated Acetone Derivatives in the Mouse Skin[a]

Chemical	Route	Dose	N[b]	S[c]	Cumulative Papillomas[d]
1,1-Dichloroacetone	Oral	200 mg/kg	40	32	1
1,1-Dichloroacetone	Topical	400 mg/kg	40	40	1
1,1,1-Trichloroacetone	Oral	50 mg/kg	40	37	1
1,1,1-Trichloroacetone	Topical	400 mg/kg	40	38	1
DMSO	Oral	0.2 ml	40	38	1
Ethanol	Topical	0.2 ml	40	38	2

[a] Chemicals were administered in a single application of 0.2 ml of the appropriate vehicle by the indicated route. Two weeks later a promotion schedule was begun with the application of 1 ug 12-O-tetradecanoyl-phorbol-13-acetate (TPA) 3 times weekly for 20 weeks.

[b] Original animals started on treatment

[c] Animals surviving to 24 weeks

[d] Cumulative numbers of papillomas observed 24 weeks after the beginning of TPA applications

Table 5. EFFECTS OF ORALLY ADMINISTERED HALOACETONITRILES ON THE DEVELOPMENT OF LUNG ADENOMAS IN A/J MICE.

Chemical	Dose[a]	Number of Animals Necropsied	% Animals with tumors	Tumors/Animal
Chloroacetonitrile	10 mg/kg x 24	28	32	0.43
Dichloroacetonitrile	10 mg/kg x 24	30	23	0.23
Trichloroacetonitrile	10 mg/kg x 24	32	28	0.38
Bromochloroacetonitrile	10 mg/kg x 24	32	31	0.34
Dibromoacetonitrile	10 mg/kg x 24	31	16	0.19
Vehicle (Emulphor 10%)	0.2 ml/mouse x 24	31	10	0.10
Ethyl Carbamate	42 mg/kg x 24	29	100	9.00

[a] Forty female strain A/J mice were administered the indicated doses of each chemical 3 times weekly for a period of 8 weeks. Treatment was begun at 10 weeks of age. Animals were sacrificed at 9 months of age.